Oxygen Stress in *Desulfovibrio vulgaris* Hildenborough: Proteomics using ITRAQ and Tandem LCMS

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Abstract

At the last annual retreat, we presented the Proteomics analysis (ICAT) of the air exposed oxygen stressed *D.vulgaris*. Even though our results in that experiment correlated with previously observed data[3] (e.g. down-regulation of genes such as *rbO*, *rbR* and the sulfate reductase pathway etc), we concluded that exposure to air had resulted primarily in cell death.

As a follow up to this, oxygen stress was monitored at much lower dosages (0.05%). We now present proteomics data of *D.vulgaris* exposed to this mild O2 stress for 240mins. We have used the newly developed ITRAQ[1] labeling system that allowed the monitoring of T0 (Control at T0) C1 (Control at 240mins) and V1 (Stressed at 240mins), in parallel. The multiplex labeling system enabled the comparison of all the samples, T0, C1 and V1. The ITRAQ label does not enrich cysteine containing peptides and therefore provides better peptide coverage for the protein hits. It must also be added that ideally both an ICAT and ITRAQ analysis would be desirable since the cysteine containing peptide enrichment (and simplification of the proteome) allows for the detection of less abundant (or in the very least a different subset) proteins.

We also used our previously optimized 2-D strategy whereby the peptides are separated first by ion exchange chromatography followed by the reverse phase separation to provide greater resolution.

Desulfovibrio vulgaris Oxygen Stress Experiment

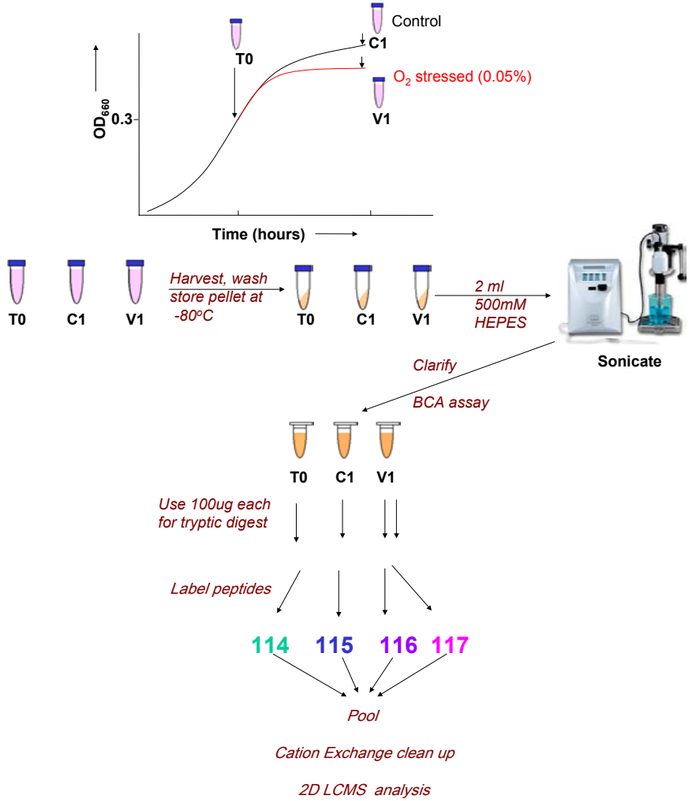
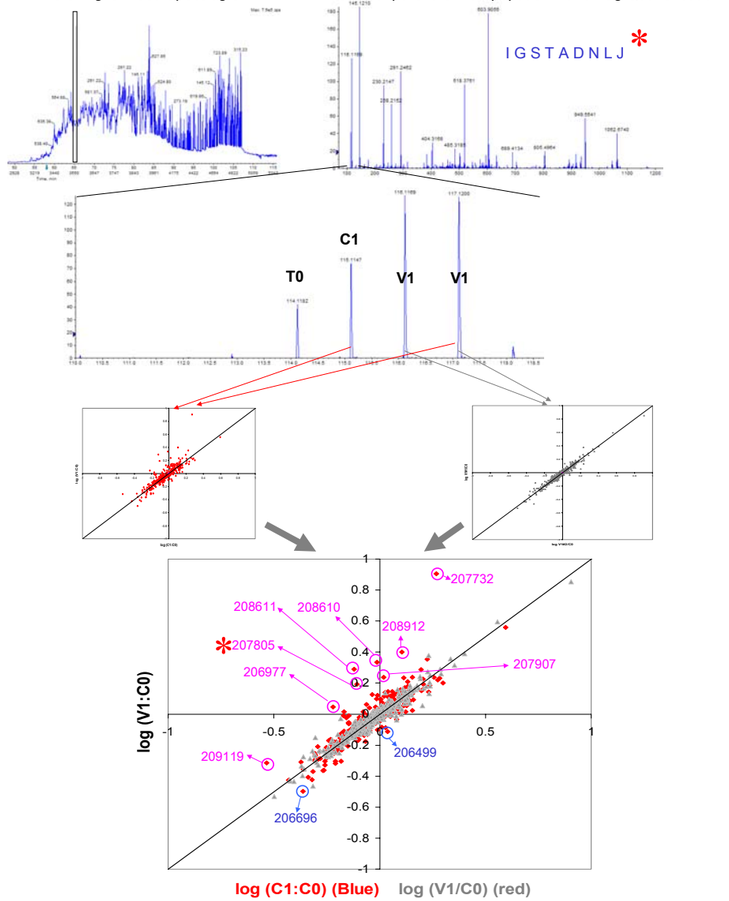


Fig.1 O_2 stress experiment and sample preparation[2]. For Proteomics, cells were sampled at two time points, T=0; before stress has been applied, and T=1 ; one doubling time (~ 240 mins) after the stress. After harvesting, cells were washed once with PBS, and the pellets stored at -80°C. Cells were lysed via sonication in 500mM HEPES at pH 8, 100µg protein per sample for tryptic digest and ITRAQ labeling. Two V1 samples were labeled with two of the four available tags to serve as an internal control. Total protein used = 400ug.

Results

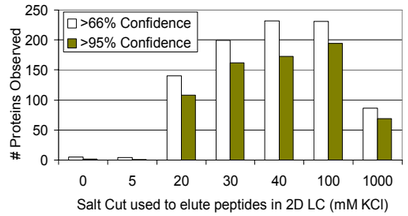
Time of flight Mass Spectrogram

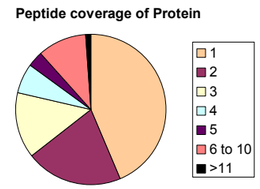
MSMS of parent ion for peptide ID and Tag Quantification.



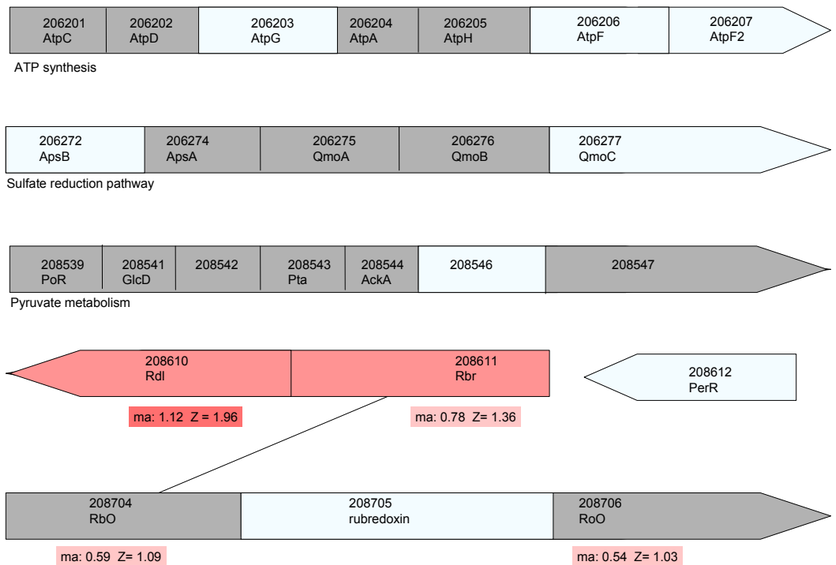
VIMSS	Name	Desc	Average Log V1 / C1
206977	NA	decarboxylase family protein	0.253756592
207732	AhpC	alkyl hydroperoxide reductase C	0.610171177
* 207805	Rbr2	rubrerythrin, putative	0.32410153
207907	NA	conserved hypothetical protein	0.189877176
208610	Rdl	rubredoxin-like protein	0.357647803
208611	Rbr	rubrerythrin	0.397368926
208912	ZraP	zinc resistance-associated protein	0.290591034
209119	NA	conserved hypothetical protein	0.191803274
206739	RpsL	ribosomal protein S12	-0.169976264
206696	NA	RNA-binding protein	-0.152229785
207257	RpsU	ribosomal protein S21	-0.121831719
206749	RpsS	ribosomal protein S19	-0.117930892
206499	NA	peptidyl-prolyl cis-trans isomerse domain protein	-0.116151279

Summary of data quality





A total of 708 proteins were identified in the 2D LCMS analysis using ITRAQ labeling technique. These 708 contain 364 unique hits. Most proteins do not show change after 240mins of exposure to 0.05% O2. Several of the upregulated proteins are known to play critical role in resistance to oxidative / aerobic stress. These include the Rbr and Rubredoxin Homologs. AhpC is known to be involved in Oxygen stress resistance in *B. fragilis*[4]. The data set includes several central operons such as the ATP synthsase, and sulfate reduction pathway that do not show any change at the protein level (see below). Proteomics data also includes several candidates where mRNA levels appear to have changed but is not reflected at the protein level.



References and Acknowledgements

1. For detailed ITRAQ methodology : refer Poster by Alyssa Redding and Applied Biosystems website
2. Biomass prepared at the Hazen lab.
3. Fournier et al., (2003). Function of Oxygen Resistance Proteins in the Anaerobic, Sulfate-Reducing Bacterium *Desulfovibrio vulgaris* Hildenborough. *J Bacteriology* 185 p71-79
4. Rocha et al (1999). Role of the Alkyl Hydroperoxide Reductase (*ahpCF*)Gene in Oxidative Stress Defense of the Obligate Anaerobe *Bacteroides fragilis*. *Bacteriology* 181 p5701-5710